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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants : Joe W. Gray et al.

Serial No.: 07/497,098 Group Art Unit: 187

Filed : March 20, 1990 Examiner: A. Marschel

For : Chromosome-Specific Staining to Detect Genetic Rearrangements

DECLARATION UNDER 37 C.F.R. SECTION 1.132

DANIEL PINKEL declares:

1. That in 1966, he graduated Phi Beta Kappa from the University of Michigan (Ann Arbor, MI), with a B.A. degree in Physics; that in 1974, he graduated from the University of California (hereinafter "U.C.") San Diego, with a Ph.D in Physics; while at U.C. San Diego from 1966 to 1974, he was an NSF Graduate Fellow and Research Assistant, and from January 1975 until June 1975, he was an Assistant Research Physicist; at U.C. Los Angeles from September 1975 until February 1977, he was an NIH Postdoctoral Fellow; from February 1977 until July 1991, he was a Biophysicist at the Lawrence Livermore National Laboratory in Livermore, California; and from July 1991 until the present, his position has been that of an Associate Professor in the Department of Laboratory Medicine at U.C. San Francisco, California.

2. That he is familiar with the information and statements within the attached press release (Attachment 1), and believes, based on his own personal knowledge, that the attributes of the invention claimed in the above-referenced application, and as set forth in said press release, are true and correct.

3. For example, he agrees with the statements by Dr. Al Deisseroth of the University of Texas M.D. Anderson Cancer Center in Houston, Texas, who stated in reference to chromosome-specific painting: "This technology is an important advance and will enable researchers to better understand the genetic basis of cancer. . . With the ability to identify the genetic changes in evolving cancer and leukemia cells, along with the relationship of these changes to cell behavior, for the first time we will be able to identify and then direct therapy to specific targets . . . . Current analytical techniques are too work intensive and the use of patient survival as a measuring point of therapy takes too long . . . . The Whole Chromosome Paints can help develop much more efficient ways to analyze the results of therapy." Thus, Dan Pinkel declares that chromosome-specific painting is a much quicker and less laborious way of detecting genetic rearrangements than the methods of the prior art.

4. Also, he agrees with Dr. Robert Jenkins, co-director of the Mayo Clinic's Cytogenetics Laboratory, who anticipates that chromosome-specific painting will make diagnoses of leukemia "much easier" than prior art diagnostic methods, and

who stated: "This technology may well be used routinely within a few years, and we could end up staining every bone marrow specimen we examine in the Mayo Clinic in this way." Further, Dr. Pinkel has no reason to doubt Dr. Jenkins' prediction that the chromosome-specific painting technology may well become the "gold standard" in identifying several types of leukemias and other genetic abnormalities.

5. Further, Dr. Pinkel agrees with the statements of Dr. Jenkins and Dr. Gordon Dewald, the latter also of the Mayo Clinic, who have employed chromosome-specific painting to count chromosomes and have found the painting probes to "vastly increase" their ability to detect chromosome abnormalities. Drs. Jenkins and Dewald indicated that whereas current leukemia detection techniques permit only about 10 percent of metaphase cells from bone marrow specimens to be analyzed, chromosome-specific paints allow "nearly every metaphase" cell to be studied. Further, he agrees with Dr. Jenkins' statements concerning the fact that researchers who had only been able to locate perhaps, for example, 20 usable samples in about 4 hours, are now, because of chromosome-specific painting, able to obtain data on about 100 samples within 30 minutes, allowing for much more accurate analyses. Thus, Dan Pinkel declares that chromosome-specific painting is more sensitive than traditional karyotyping for the detection of abnormal cells in a patient.

6. Still further, he agrees with Dr. Jeffrey Trent of the University of Michigan Medical Center (Ann Arbor, MI), who

has employed chromosome-specific painting to study chromosome changes in common adult cancers, like breast cancer and melanoma, and has stated: "Solid cancer tumors are extremely complex genetically, and these paints may well help define the genetic alterations that are important in these disorders."

7. Dan Pinkel further declares that he is familiar with the information and statements in the attached article and inset from Science, 254: 378-379 (18 October 1991) (Attachment 2), and believes, based on his own personal knowledge, the following concerning those materials. He believes that the publication of the article concerning chromosome-specific painting research by Integrated Genetics and the inset referring to Joe Gray's and his work with chromosome-specific painting is further evidence that the use of chromosome-specific painting is widespread, and is expected to have a major impact on medicine and biology. Further, the article and inset are evidence of the commercial success of chromosome-specific painting. One of the first goals of the recently founded company Imagenetics was the commercialization of the chromosome-specific painting technology. As noted in the article on "FISHing" (FISH is an acronym for fluorescent in situ hybridization), the waiting time for the results of amniocentesis can be cut from three weeks to less than two days wherein most of the delay with previous techniques "comes from the time it takes to grow fetal cells in culture so that a sufficient number of them will be in metaphase" (FISHing article, column 1, paragraph 2). Dan Pinkel declares that a goal

for chromosome-specific painting had been to obtain information rapidly from low quality metaphase chromosomes and from interphase nuclei. As indicated in the FISHing article and in the press release, that goal has been realized.

8. Further, Dan Pinkel declares that the FISHing article explains that the standard prior art approach, that is, chemically stained bands on high quality metaphase spreads, is labor intensive, time consuming and requires a highly trained analyst, and further does not allow the detection of structural aberrations involving less than 3-15 million bases (megabases). Further, conventional banding analysis cannot be used on interphase nuclei. This invention represents a pivotal change in the concept of banding analysis from the inflexibility of chemical staining to the flexibility of probe-produced staining wherein the staining pattern based upon nucleic acid sequence can be adjusted by investigators for particular purposes. Joe Gray's and his accomplishment of initially painting entire chromosomes indicated the feasibility of focusing on chromosomal subregions with ever increasing precision and flexibility as appropriate collections of DNA sequences were assembled.

9. Dan Pinkel further declares that the FISHing article from column 3 on page 378 to the final column of the article on page 379 is evidence that the claimed noninvasive technique of chromosome-specific painting chromosomal material of fetal cells obtained from maternal blood, works.

10. Dan Pinkel declares that at the time he and Joe Gray began work with chromosome-specific painting with high complexity probes, the rest of the research community was concentrating on using in situ hybridization to map short cloned sequences. Only occasionally and with great effort, was in situ hybridization used to obtain some information about the position of a lesion, for example, a breakpoint relative to a particular DNA sequence. At that time, conventional banding analysis with various chemical stains was used for chromosome identification and detection of abnormalities. Among the uses of such conventional banding analysis were prenatal diagnosis, analysis of chromosomes from abnormal individuals, and gathering information on the chromosomal abnormalities associated with various malignancies. Detection of chromosome translocations due to exposure of humans to environmental hazards, such as, chemicals and radiation, while recognized as highly desirable, could not be widely applied due to the time and skill required for analysis. In addition to those health-related applications, a great deal of biological research involved banding analysis of chromosomes from humans and other species. As indicated above, conventional banding analysis has severe limitations such as the requirement of preparing high quality metaphase spreads to make the chemical staining interpretable. Joe Gray's and his goal was to expand dramatically the use of in situ hybridization and convert it from a method of mapping probes to a primary method of

staining chromosomes. The staining is based upon nucleic acid sequence of each portion of the genome.

11. Dan Pinkel declares that professional approval and acceptance of Joe Gray's and his invention of chromosome-specific painting is evidenced by the many others who are now working with chromosome-specific painting, and the number of publications concerning chromosome-specific painting in the premier, peer reviewed journals, such as, Science, Nature and PNAS, among others. Such publications are evidence of the importance that the scientific community places on chromosome-specific painting. Use of the chromosome-specific painting technology has grown explosively since Joe Gray and he initially demonstrated its feasibility.

12. Dan Pinkel still further declares that because of Joe Gray's and his belief, as well as that of their informed colleagues, that chromosome-specific painting was such a dramatic step beyond the state of the art at the time it was invented, that peer review committees for grant applications would not accept the calculations and plausibility arguments that were motivating them. Therefore they delayed applying for grants until they had actually reduced to practice the painting of an entire chromosome and had photographic proof to enclose with their grant application. They figured that a photograph was worth more than thousands of words of plausibility arguments.

The undersigned declares further that all statements made herein are of his own knowledge, are true, and that all

statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Dated: 10-31-91

DANIEL PINKEL, Ph.D